

# The Use of Latex Agglutination for the Diagnosis of Acute Human Leptospirosis

Sukone Pradutkanchana, MSc\*,  
Janya Nakarin, PhD\*\*

\* Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla

\*\* Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla

---

The authors evaluated a simple latex agglutination test for the serodiagnosis of acute human leptospirosis. A total of 380 serum samples from 85 confirmed leptospirosis patients and 202 non-leptospirosis patients were selected. Using the selected cut-off value of weakly positive, the overall sensitivity and overall specificity of the test were 94.1% and 97.0% respectively. The weighted kappa value for agreement reading between two independent examiners was 0.82. When focused on the first sera obtained from the patients, the sensitivity of the test for acute infection sera was only 17.6%. The latex agglutination test is easy to perform with no need for training and no requirement of special equipment. The assay has a good sensitivity and specificity. This is an interesting alternative assay for serodiagnosis of acute human leptospirosis.

**Keywords:** Leptospirosis, Evaluation, Latex agglutination

**J Med Assoc Thai 2005; 88 (10): 1395-400**

**Full text. e-Journal:** <http://www.medassocthai.org/journal>

---

Leptospirosis is a zoonosis with a worldwide distribution, caused by *Leptospira interrogans*<sup>(1)</sup>. As the clinical symptoms and signs of leptospirosis often are nonspecific, the disease is easily mistaken for other acute febrile illnesses including dengue fever, scrub typhus, and murine typhus<sup>(2,3)</sup>. Laboratory testing to confirm the clinical diagnosis is essential for optimal treatment and patient management. The laboratory diagnosis of leptospirosis mainly depends on culture<sup>(4)</sup> and serological method for detection of specific antibody<sup>(5)</sup>. Both culture and the reference method, microscopic agglutination test (MAT), seem to be laborious as well as expensive. Several methods for detecting specific IgM antibody including, enzyme-linked immunosorbent assay (ELISA)<sup>(6,7)</sup>, Dipstick<sup>(8)</sup>, indirect immunofluorescent assay<sup>(9)</sup> are widely used and some of them are now available commercially. None of these tests are easy to perform, all require special and expensive equipment and can be used only by trained personnel. Thus, there is a need to develop a conven-

tional method that is rapid, simple with high sensitivity and specificity and that can be used in small health centers or in developing countries where expensive equipment is lacking. In the present study the authors evaluated a simple latex agglutination test for detecting leptospiral antibodies for use as a rapid diagnostic method with no requirement for specialized equipment.

## Material and Method

### Study populations

Serum samples were obtained from patients admitted to Songklanagarind and Hat Yai Hospitals. These are two major tertiary teaching hospitals serving the 200,000 inhabitants of Hat Yai city which is 930 kilometers to the south of Bangkok in Thailand. One hundred and seventy-eight sera from 85 patients who had an acute fever of >38 °C for more than 1 day but not more than 3 weeks were collected. Exclusion criteria were the presence of profuse rhinorrhea, exudative pharyngitis, pneumonia, urethritis and diarrhea. All sera were assayed for leptospiral antibody by the microscopic agglutination test (MAT). Only those sera from patients with at least two separate serum samples that showed a four-fold or greater increase in MAT titer

---

Correspondence to : Pradutkanchana S, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat-Yai, Songkla 90112, Thailand. Phone: 0-7445-1585, Fax: 0-7421-2908, E-mail: [psukone@ratree.psu.ac.th](mailto:psukone@ratree.psu.ac.th)

were included in the present study. In addition, the background of leptospiral antibody was determined from 100 healthy blood donors, 20 patients with syphilis (fluorescent treponema antibody-absorbed test with positive result of 3+ to 4+), 20 patients with anti-nuclear antibody IFA positive titer 1:1,280 and 62 patients with diseases commonly confused with leptospirosis. These patients consisted of 20 with scrub typhus (IFA titer against *Orientia tsutsugamuchi*  $\geq$  1:400)<sup>(10)</sup>, 22 with murine typhus (IFA titer against *Rickettsia typhi*  $\geq$  1:400)<sup>(11)</sup> and 20 with dengue fever (hemagglutination inhibition antibody titer against dengue virus  $\geq$  1:2,560)<sup>(12)</sup>. All sera were kept at -80 C until used.

#### **Antigen preparation**

Leptospire of serovar Bataviae were grown in neopeptone liquid medium (Difco Laboratories, USA) which contained 10% young rabbit serum (Biochrom AG, Germany). Cultures were kept at room temperature for 5-7 days. The organisms were observed using dark field microscopy (OLYMPUS model BH-2). Leptospiral cultures of approximately 3+ to 4+ with active movement and no clumping were suitable for use. After washing leptospire with phosphate buffered saline twice by centrifugation at 10,000 g at 4 C for 10 minutes each, leptospire were sonicated in an ice bath at a setting of 20 kHz, for 30 seconds each time and a total time of 5 minutes. Aliquots of the sonic extract were stored at -80 C

#### **Microscopic agglutination test (MAT)**

MAT was performed with a panel of live leptospire as previously described by Galton *et al*<sup>(5)</sup>. The panel consisted of the following 23 serovars: Australis, Ballico, Bratislava, Akayami, Rachamati, Bataviae, Canicola, Cellidoni, Djasiman, Grippotyphosa, Hebdomadis, Hyos, Tarassovi, Icterohemorrhagiae, Copenhageni, Javanica, Saigon, Pomona, Pyrogenes, Sejroe, Hardjo, Wolffi and Andamana. An agglutination was performed in a microtiter plate at a serum dilution starting at 1:100 by adding 25  $\mu$ l of 1:50 diluted serum and 25  $\mu$ l of each live leptospira serovar. The mixtures were then mixed gently. After leaving at room temperature for 2-3 hours, 3  $\mu$ l of the suspension was dropped on to a slide. The agglutination was observed using dark field microscopy (OLYMPUS model BH-2) at a final magnification of 100X. Any serum specimen giving a positive reaction was then retested against the relevant serovars to determine the endpoint titer which was the highest dilution giving more than 50 per cent agglutination of leptospire.

#### **Latex agglutination assay**

After washing carboxylated-modified polystyrene latex beads (Sigma-Aldrich, USA) with phosphate buffer saline pH 7.2, an optimal concentration of sonicated leptospiral antigen was adsorbed to the latex particles for 1 hour. Antigen coated latex particles were then washed 3 times with phosphate buffered saline pH 7.2 then resuspended to give a 3% latex suspension in 0.5% bovine serum albumin in phosphate buffered saline. The test was performed by placing 5  $\mu$ l of serum sample on a white agglutination card. Subsequently, the serum was mixed with an equal volume of coated latex particles. The card was rotated gently for 5 min and read for the aggregation of the latex particles on the scale 0 to 3+. Positive and negative controls were included with every batch tested.

#### **Statistical analysis**

The sensitivity, specificity, false positive rate, false negative rate, negative predictive value and positive predictive value of the assays were calculated according to the method described by Griner<sup>(13)</sup>. The weighted kappa statistical value for agreement between the reading from two different examiners and the receiver operating characteristic (ROC) curves that plotted between true positive versus false positive rates were determined using the Statistical Packages for the Social Science release 9.05 (SPSS, Chicago, USA).

#### **Results**

A total of 380 serum samples from 85 MAT confirmed leptospirosis patients and 202 non-leptospirosis patients were evaluated using the latex agglutination test for diagnosis of acute human leptospirosis. Using ROC analysis, the sensitivity and specificity of the latex agglutination test was 94.1% and 97.0%, respectively at the selected cut-off point of weakly positive. The area under ROC of the test was 0.97 and the weighted kappa value was 0.82. However, the overall sensitivity and overall specificity are shown in Table 1. When acute phase sera from patients diagnosed with leptospirosis was considered, the sensitivity of the first sera obtained from the patients was only 17.6%. The cross reaction assessment was performed with serum from healthy blood donors, patients with confirmed syphilis, auto immune disease and various diseases commonly confused with leptospirosis such as scrub typhus, murine typhus and dengue fever. The backgrounds of leptospiral antibody by latex agglutination are shown in Table 2. When using a cut-off value of

**Table 1.** The sensitivity and specificity of the latex agglutination test for diagnosis of acute human leptospirosis

Cut-off value	Overall sensitivity*	%	Overall specificity**	%	Sensitivity of the first sera obtained from patients*	%
Positive 3+	2/85	2.4	202/202	100.0	0/85	0.0
Positive 2+	28/85	32.9	202/202	100.0	2/85	2.4
Positive 1+	77/85	90.6	201/202	99.5	13/85	15.3
Weakly positive	80/85	94.1	196/202	97.0	15/85	17.6
Negative	85/85	100.0	0/202	0.0	85/85	100.0

\* No of positive sera/No of sera tested

\*\* No of negative sera/No of sera tested

**Table 2.** The background of leptospiral antibody by latex agglutination test for diagnosis of acute human leptospirosis

Agglutination result	Negative control group (n)					
	Healthy blood donors	Scrub typhus	Murine typhus	Dengue fever	Syphilis	ANA positive sera
Negative	99	17	21	19	20	20
Weakly positive	1	2	1	1	0	0
Positive 1+	0	1	0	0	0	0
Positive 2+	0	0	0	0	0	0
Total	100	20	22	20	20	20

Note: ANA = anti nuclear antibody

**Table 3.** The statistical performances of the latex agglutination test for diagnosis of acute human leptospirosis

Statistical values	Percent (%)
Sensitivity	94.1
Specificity	97.0
False positive rate	3.0
False negative rate	5.9
Positive predictive value	93.0
Negative predictive value	97.5
Accuracy	96.2

weakly positive, the statistical performances of the latex agglutination test are summarized in Table 3.

### Discussion

The antigen used for coating the latex particles was prepared from *Leptospira interrogans* serovar Bataviae while the antigens used in the commercial kits for detecting leptospiral antibody were extracted from *Leptospira biflexa* serovar Patoc which is a non-pathogenic serovar and has broad cross reactivity. A

previous study reported that the use of antigen from the most common infective serovar for the tests in endemic areas was more sensitive and the reaction between patients' sera and the antigen was stronger than when antigen from a nonpathogenic serovar was used<sup>(14)</sup>. In the present study, the authors used sonicated antigen from serovar Bataviae because this serovar is the most common in Thailand, especially in the southern region of Thailand where the study site was located<sup>(15)</sup>. Another study in Thailand used antigen from serovar Pyrogenes<sup>(16)</sup> which is most common in the north-eastern region of Thailand<sup>(17)</sup>.

The reading results from two independent observers showed almost perfect agreement with a weighted kappa statistical value of 0.82. This indicated that no special training was required to perform the latex agglutination test. Most reading scores given by the two observers were the same for any patients' sera and no result of any serum differed by more than one dilution.

In the present study the sensitivity of the latex agglutination test was relatively high. Using the selected cut-off value of weakly positive, the overall sensitivity of the test was 94.1%. This was higher than

found in previous studies from multicenters in 4 countries. These gave a mean sensitivity of 82.3% (range from 76.5-83.3)<sup>(18)</sup>, however a previous report in Thailand gave a sensitivity of 94.7%<sup>(16)</sup>. This might be due to the use of different sources of pathogenic strain of leptospire. Smits *et al*<sup>(18)</sup> used antigen from serovar Hardjo which is the most common in cattle as a carrier and not frequently reported in outbreaks in Thailand, while Naigowit *et al*<sup>(16)</sup> used serovar Pyrogene as a source of antigen. However, an evaluation of the use of serovar Pyrogenes using a latex agglutination test supplied from National Institute of Health, Ministry of Public Health, Thailand and the serum samples obtained from the southern region of Thailand where the outbreak is caused mainly by serovar Bataviae revealed that the sensitivity of the test was only 83.1%<sup>(19)</sup>.

When the first sera obtained from the patients were considered, the sensitivity of acute serum was only 17.6%. This is relatively low when compared to previous reports that gave values of nearly 40% sensitivity<sup>(16,18)</sup>. A previous report found that using serum collected during the first 10 days the sensitivity of the latex agglutination test was not much different from that of the microscopic agglutination test (MAT)<sup>(18)</sup>. In contrast another study reported that the sensitivity of acute serum by the MAT was as low as 12.6%<sup>(16)</sup>. This discrepancy may be related to the different diagnostic criteria used for the diagnosis of leptospirosis. Smits *et al*<sup>(18)</sup> defined a positive case of leptospirosis disease by obtaining either a positive culture from blood or urine or a MAT titer of  $\geq 1:160$  obtained from one or more samples. Naigowit *et al*<sup>(16)</sup> used a cut-off value of MAT at  $\geq 1:400$  in single serum or when there was a four-fold increase in the titer of paired serum. In the present study the authors found that the sensitivity of the MAT was very low at 1.2% (data not shown). The case definition of acute leptospirosis for the present study was only that patients had paired serum samples showing a four-fold or greater rise in MAT titer. Serum samples with high MAT antibody titer that persisted in acute serum with less than a four-fold rise in titer of convalescent serum were not included in the present study.

The high specificity of the latex agglutination test was confirmed by testing 202 samples from healthy blood donors and the patients with syphilis, autoimmune diseases and the diseases commonly confused with leptospirosis including scrub typhus, murine typhus and dengue hemorrhagic fever. The mean specificity of latex agglutination test was 97.0%, slightly higher than previous studies<sup>(16,18)</sup>. Only 6 out of the

202 sera tested showed aggregation of latex particles and most of those were weakly positive. Among these 6 false positive cases, scrub typhus accounted for 3 (50%) of cases. It is interesting that scrub typhus is usually a major cause of a false positive result in an immunodiagnostic test of leptospirosis<sup>(9)</sup> and leptospirosis usually has cross reactivity in the serodiagnosis of scrub typhus *vice versa*<sup>(20)</sup>. The reasons for this are unclear, but these two organisms may probably share a common antigen or previous exposure to leptospire in patients with scrub typhus may occur.

The latex agglutination test is extremely simple and rapid to perform. The result can be read within 5 minutes, compared to 3 hours using the dipstick assay<sup>(21)</sup> or more than 1 hour with an ELISA test<sup>(22)</sup>. The aggregation of latex particle is not difficult to read, the examiner can perform the test without the need for training. Special equipment is not required. The assay gives a reliable result with a good sensitivity and specificity. This is an interesting alternative test for diagnosis of acute human leptospirosis especially in small health care units or in developing countries where sophisticated equipment is lacking.

#### Acknowledgement

This work was supported by a grant from the National Research Council of Thailand. The authors wish to thank Dr. Metta Ongsakul and Dr. Brian Hodgson for reviewing the manuscript.

#### References

1. Farr RW. Leptospirosis. Clin Infect Dis 1995; 21: 1-8.
2. Faine S. Guidelines for the control of leptospirosis. WHO, Geneva, Switzerland. 1982.
3. Pradutkanchana J, Pradutkanchana S, Kemapananus M, Woothipom N, Silpapojakul K. The etiologies of acute pyrexia of unknown origin in children after a sudden severe flood. Southeast Asian J Trop Med Public Health 2003; 34: 175-8.
4. Sundharagiati B, Boonpaknavig S, Panasampol K, Harinasuta C. Leptospirosis in rat of Pitsanuloke and Chiangmai provinces. J Med Assoc Thai 1964; 47: 680-4.
5. Galton MM, Powers DK, Hall AM, Cornell RG. A rapid microscopic slide screening test for the serodiagnosis of leptospirosis. Am J Vet Res 1958; 19: 505-12.
6. Petchclai B, Kunakorn M, Hiranras S, Potha U, Liemsuwan C. Enzyme-linked immunosorbent assay for leptospirosis immunoglobulin M specific antibody using surface antigen from a patho-

- genic *Leptospira*: A comparison with indirect hemagglutination and microagglutination tests. *J Med Assoc Thai* 1992; 75(Suppl 1): 203-8.
7. Cumberland P, Everard COR, Levett PN. Assessment of the efficacy of an IgM-ELISA and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. *Am J Trop Med Hyg* 1999; 61: 731-4.
  8. Gussenhoven GC, van der Hoorn MAWG, Goris MGA, Terpstra WJ, Hartskeerl RA, Mol BW, et al. LEPTO Dipstick, a dipstick assay for detection of *Leptospira* specific immunoglobulin M antibodies in human sera. *J Clin Microbiol* 1997; 35: 92-7.
  9. Pradutkanchana S, Pradutkanchana J, Khuntikij P. Detection of IgM specific antibody using indirect immunofluorescent assay for diagnosis of acute leptospirosis. *J Med Assoc Thai* 2003; 86: 641-6.
  10. Pradutkanchana J, Silpapojakul K, Paxton H, Pradutkanchana S, Kelly DJ, Strickman D. Comparative evaluation of four serodiagnostic tests for scrub typhus in Thailand. *Trans R Soc Trop Med Hyg* 1997; 91: 425-8.
  11. Silpapojakul K, Pradutkanchana J, Pradutkanchana S, Kelly DJ. Rapid and simple serodiagnosis of murine typhus: comparison of the dot enzyme-linked immunosorbent assay with the latex agglutination and the Weil-Felix test. *Trans R Soc Trop Med Hyg* 1995; 89: 625-8.
  12. Clarke DH, Casals J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am J Trop Med Hyg* 1958; 7: 561-73.
  13. Griner PF, Mayewsk RJ, Muslin AI. Selection and interpretation of diagnostic tests and procedures: Principles and applications. *Ann Intern Med* 1983; 94: 553-600.
  14. Petchclai B, Srisarin A, Potha U, Hiranras S, Wongpaitoon V. Evaluation of two screening tests for human leptospirosis. *J Med Assoc Thai* 1990; 73: 64-7.
  15. Pradutkanchana S, Pradutkanchana J, Kanjanapin W, Siripaitoon P. An outbreak of leptospirosis after severe flood in Hat Yai in 2000. *J Infect Dis Antimicrob Agents* 2002; 19: 9-13.
  16. Naigowit P, Leupaktra O, Yasang S, Biklang M, Warachit P. Development of a screening method for serodiagnosis of leptospirosis. *Intern Med J Thai* 2001; 17: 182-7.
  17. Montian-arsana S, Kusum M, Naigowit P, Kamaswat S. Epidemics of leptospirosis in north eastern province of Thailand in 1996. *J Health Sci* 1997; 6: 241-8.
  18. Smits HL, van der Hoorn MAWG, Goris MGA, Gussenhoven GC, Yersin C, Sasaki DM, et al. Simple latex agglutination assay for rapid serodiagnosis of human leptospirosis. *J Clin Microbiol* 2000; 38: 1272-5.
  19. Kemapanmanus M, Sretrirutchai S, Khuntikij P, Pradutkanchana S, Pradutkanchana J. A prospective evaluation of four immunodiagnostic assays of human leptospirosis. *Southeast Asian J Trop Med Public Health* 2004; 35: 863-7.
  20. Brown GW, Shirai A, Rogers C, Groves MG. Diagnostic criteria for scrub typhus: probability values for immunofluorescent antibody and *Proteus* OX-K agglutinin titres. *Am J Trop Med Hyg* 1983; 33: 311-5.
  21. Sehgal SC, Sharma VS, Sugunan AP. LEPTO Dipstick: a rapid and simple method for serodiagnosis of acute leptospirosis. *Trans R Soc Trop Med Hyg* 1999; 93: 161-4.
  22. Winslow WE, Merry DJ, Pirc ML, Devine PL. Evaluation of a commercial enzyme-linked immunosorbent assay for detection of immunoglobulin M antibody in diagnosis of human leptospiral infection. *J Clin Microbiol* 1997; 35: 1938-42.

---

## การใช้การทดสอบลาเท็กซ์แอกกูตินเนชั่นในการตรวจวินิจฉัยโรคเลปโตสไปโรซิสชนิดเฉียบพลัน

สุคนธ์ ประดุจกาญจนา, จรรยา นครินทร์

คณะผู้วิจัยได้ทำการประเมินการทดสอบวิธีลาเท็กซ์แอกกูตินเนชั่นสำหรับตรวจวินิจฉัยผู้ป่วยโรคเลปโตสไปโรซิสชนิดเฉียบพลัน โดยทดสอบกับซีรัมจำนวน 380 ตัวอย่างจากผู้ป่วยโรคเลปโตสไปโรซิสจำนวน 85 ราย และไม่ใช่โรคเลปโตสไปโรซิสอีก 202 ราย เมื่อเลือกใช้ค่าจุดตัดที่เหมาะสมที่ระดับ weakly positive พบว่าค่าความไวรวม และความจำเพาะรวมของการทดสอบเท่ากับร้อยละ 94.1 และ 97.0 ตามลำดับ ค่าทางสถิติ kappa ซึ่งใช้ประเมินความเหมือนของการอ่านผลการทดสอบระหว่างผู้ปฏิบัติงานสองท่านมีค่าเท่ากับ 0.82 สำหรับการทดสอบในซีรัมครั้งแรกที่เจาะจากผู้ป่วยพบว่ามีค่าความไวเท่ากับร้อยละ 17.6 วิธีลาเท็กซ์แอกกูตินเนชั่น เป็นวิธีที่สามารถทำการทดสอบได้ง่าย ไม่จำเป็นต้องฝึกอบรมหรือใช้เครื่องมือราคาแพง ผลการทดสอบมีค่าความไวและความจำเพาะสูง ดังนั้นวิธีนี้จึงเป็นทางเลือกใหม่ที่น่าสนใจสำหรับใช้ตรวจวินิจฉัยโรคเลปโตสไปโรซิสชนิดเฉียบพลันในห้องปฏิบัติการ

---